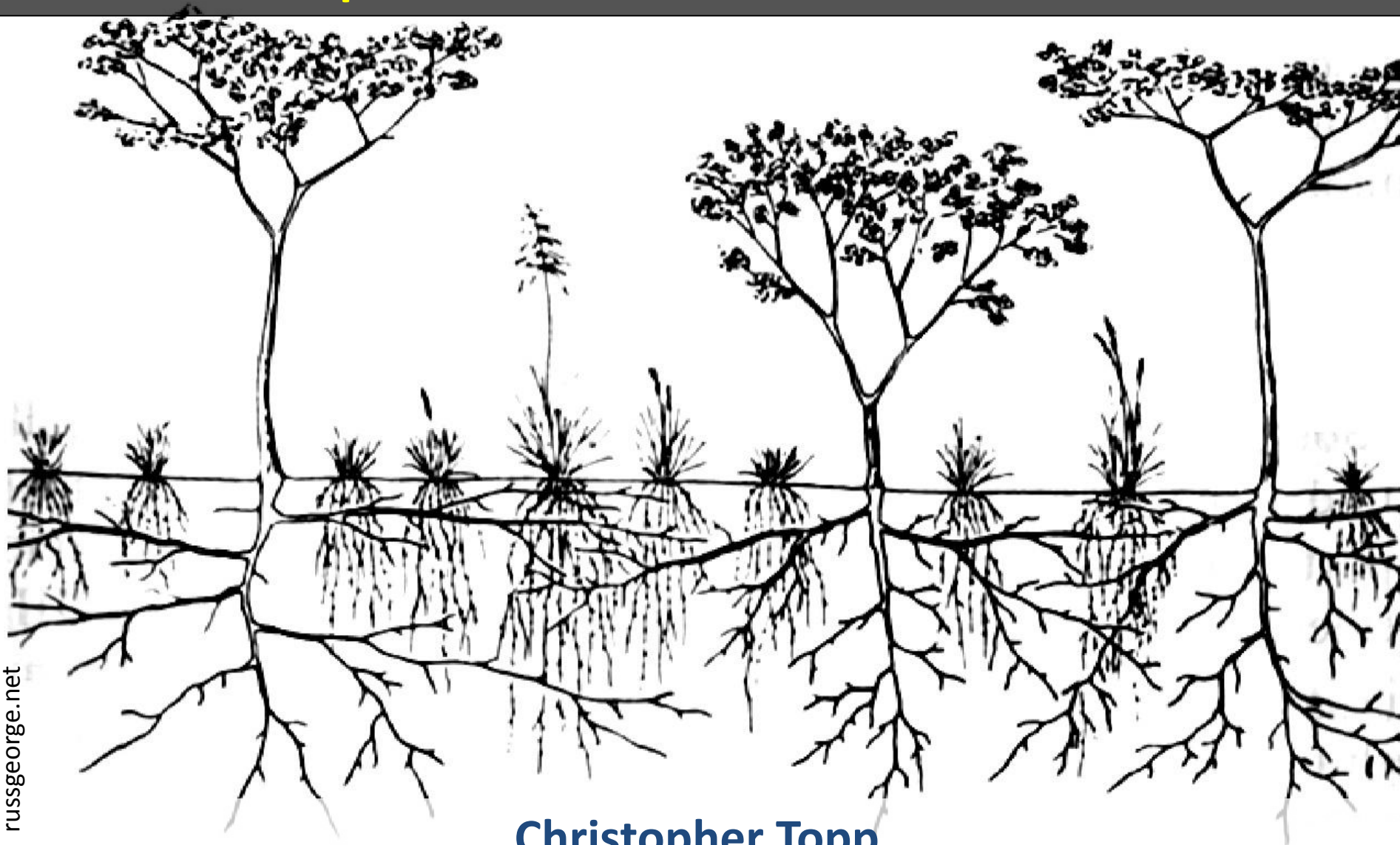


# Towards an integrated understanding of the plant and plant-environment interactions



**Christopher Topp**

Donald Danforth Plant Science Center

# How do we study the plant as an integrated system?





# How do we study the plant as an integrated system?



**root and shoot phenotyping in controlled environments**

**Todd Mockler and Topp Labs**

# Plant phenomics is relatively nascent; we lack expertise in tool development, data processing and analysis

Two groups we need to forge relationships with:  
**medical imaging and industrial phenotyping**

1. embed plant phenotyping at medical schools  
→ **advanced imaging tools (X-ray CT, PET, Spectroscopy, etc.)**
2. leverage advances in production agriculture for science  
→ **robotics for throughput and precision**
3. technology moves fast  
→ **focus on open source tools and flexible platforms**

# We can leverage existing technologies in industrial engineering, robotics, computer vision/AI

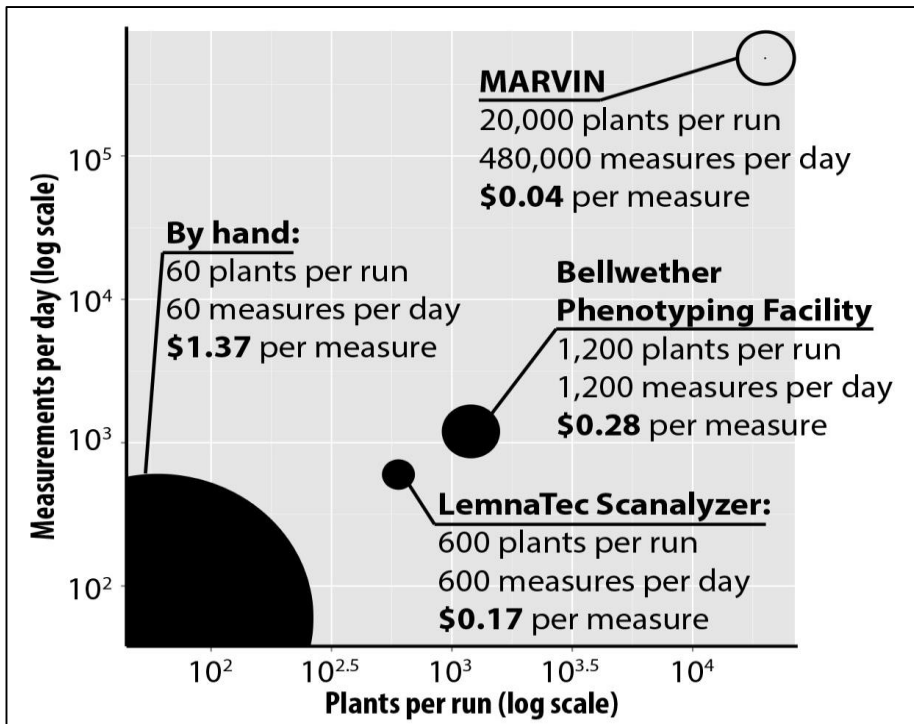


Contact: Rick van de Zedde

**MARVIN: Rick van de Zedde, Wageningen University, Netherlands**



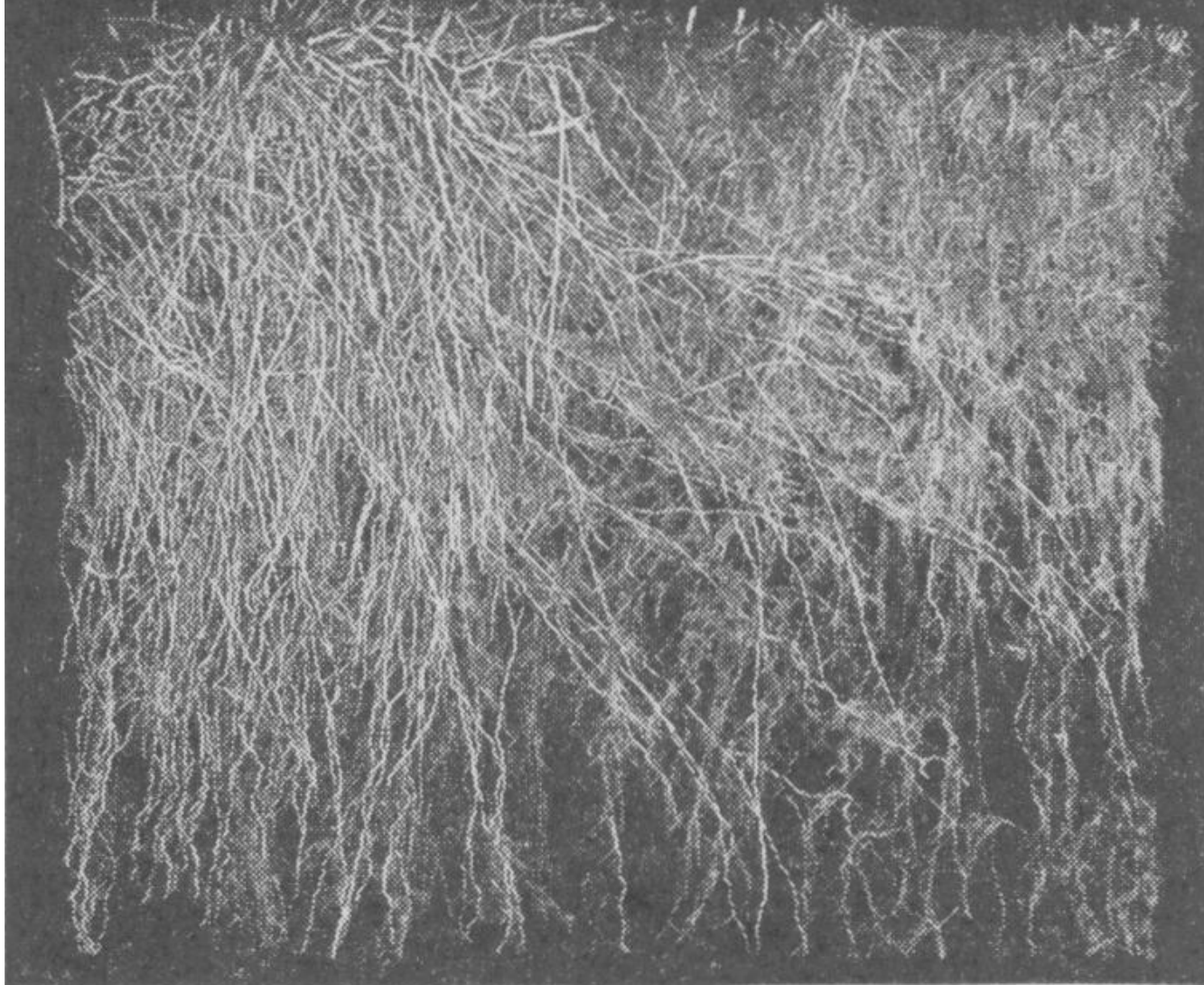
# High Throughput Phenotyping



1. Build a versatile, ultra-throughput platform capable of exploring the growth and environmental response in the world's germplasm
1. Analytic capabilities to interpret unprecedented architectural data from the root and shoot and place this into a genetic framework
2. Test specific hypotheses concerning whole plant architecture, the developmental constraints of yield and biomass accumulation, and high resolution time lapses of plant responses to stresses and competitors

# How do we study the plant as an integrated system?

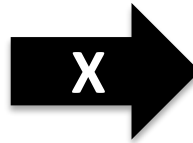
**Roots are often the bottleneck**



Weaver and Voigt Botanical Gazette 1950

# Identifying the genetic and functional basis of root architecture: **integrated root phenotyping with quantitative genetics**

Root phenotyping  
methods

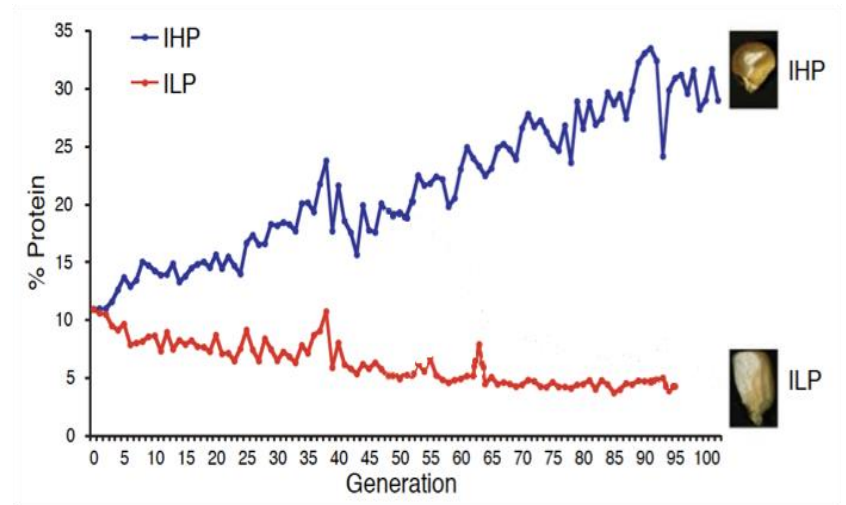


High-resolution germplasm  
with important agronomic traits

1. 3D hydroponic, gel, sand, turf surface imaging
2. PET and/or X-ray CT in pots
3. Image analysis of excavated root crowns from the field – optical 2D and X-ray 3D
4. Mini-rhizotrons, X-ray CT soil cores or other developing in situ phenotyping methods (GPR, THz)

Environmental control ↑

↓ Agricultural relativity

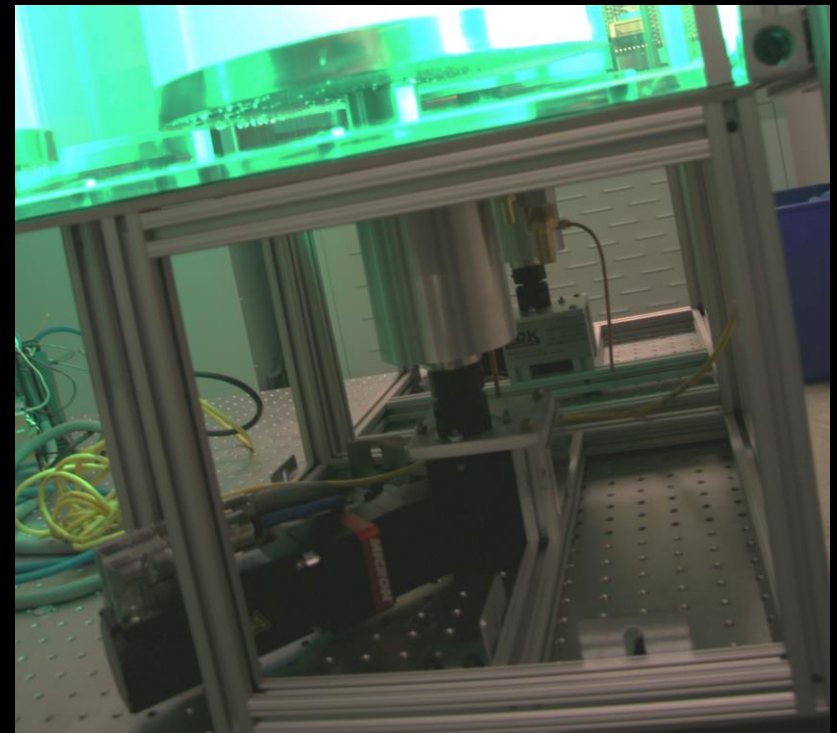
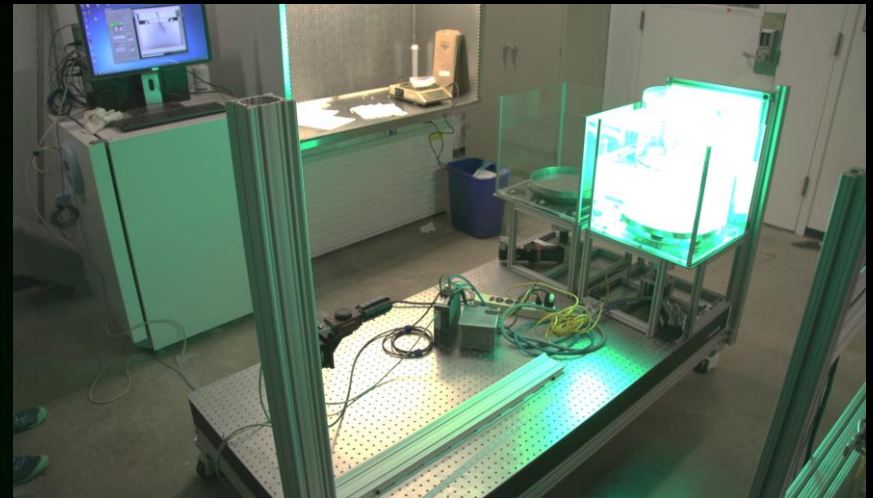
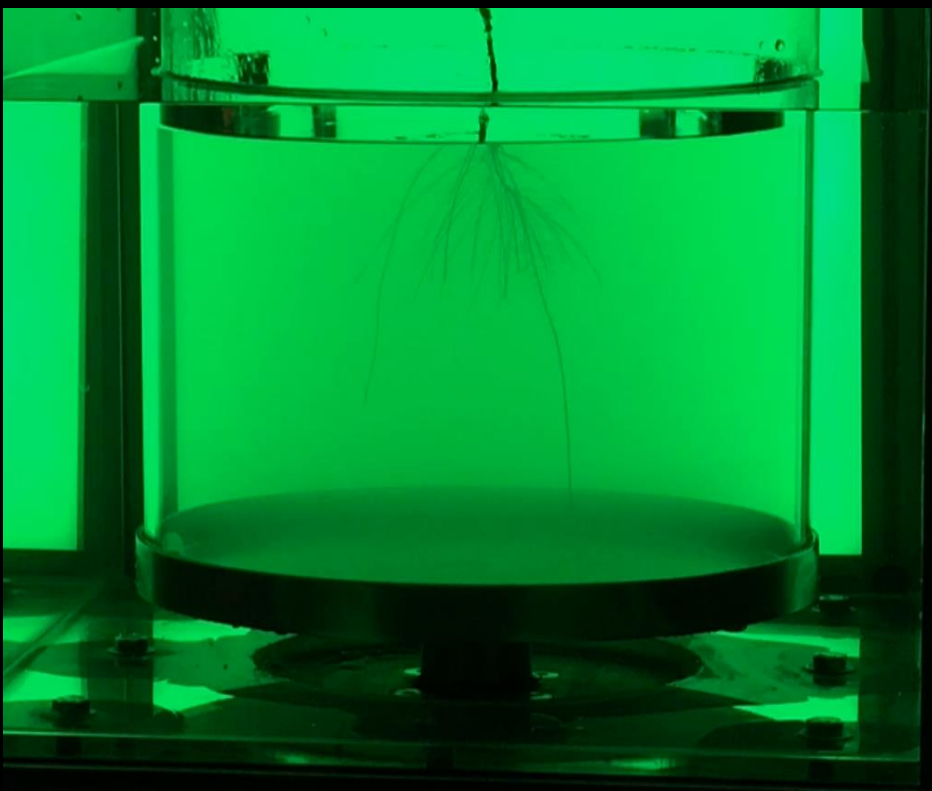




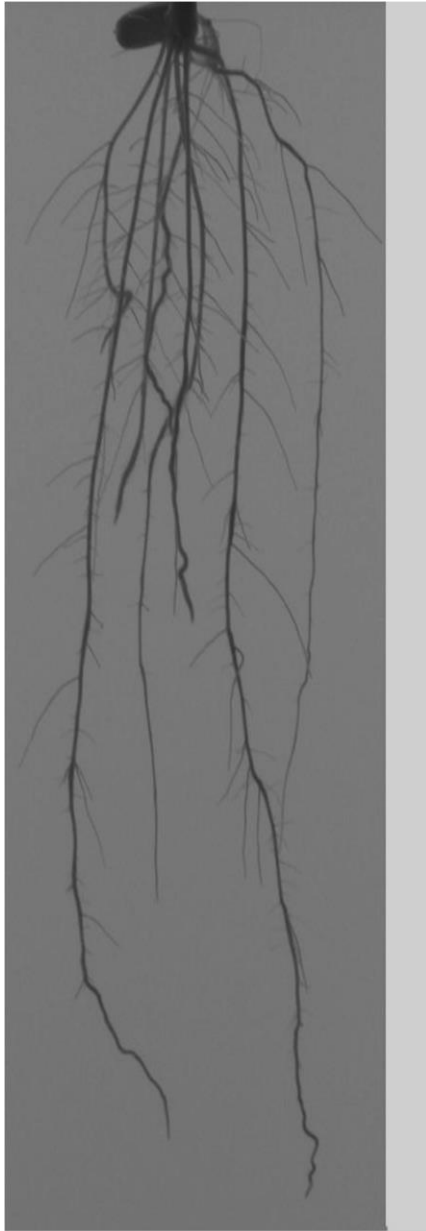
# Optical Projection Tomography (OPT) platform for root phenotyping in 3D



Imaging time: 20 seconds per plant



# 3-dimensional modelling of root architecture



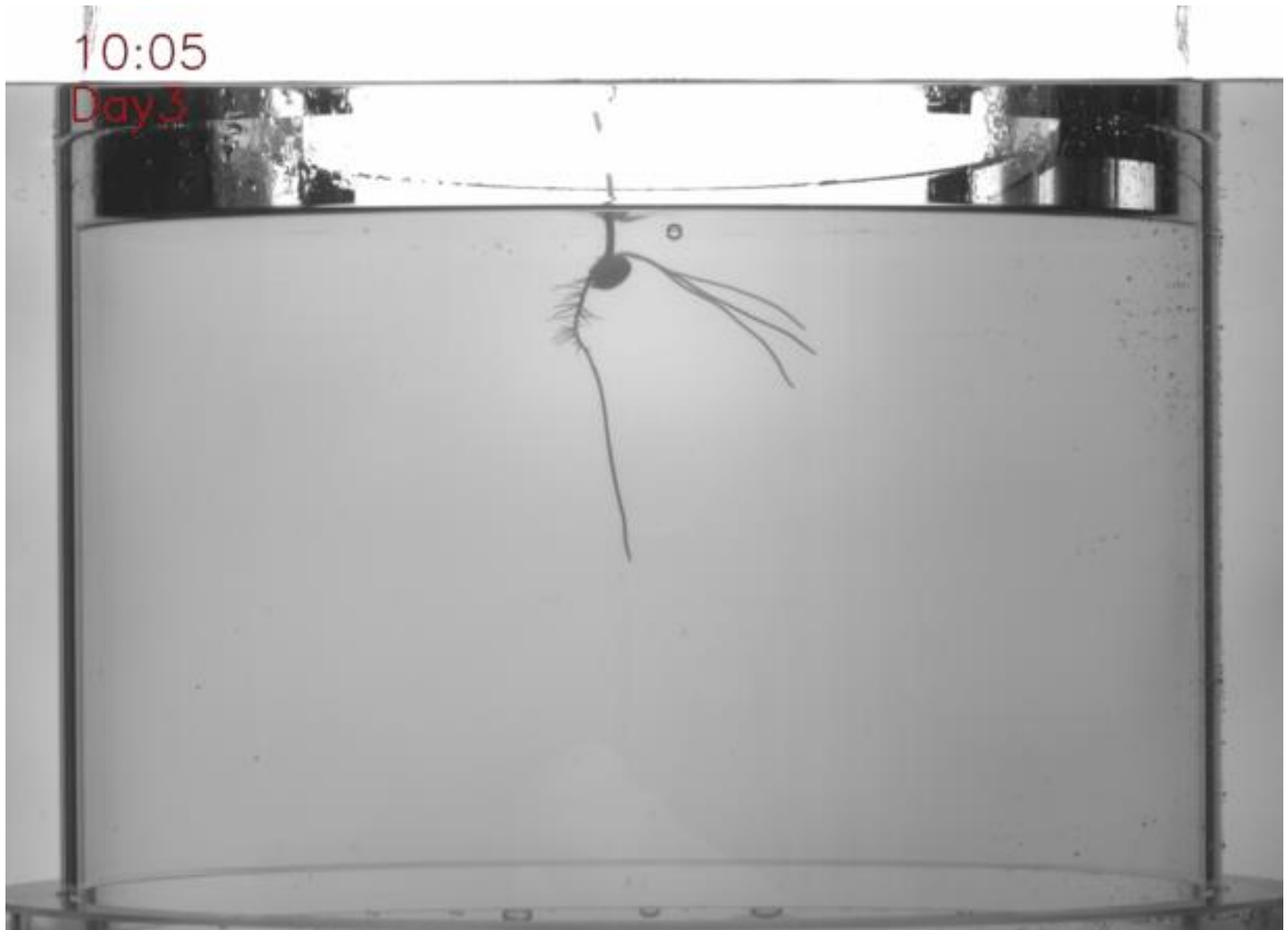
## traits analyzed in 3D

1. median root number
2. maximum root number
3. root system volume
4. convex hull volume
5. solidity
6. surface area
7. bushiness
8. total root length
9. root system volume
10. specific root length
11. number of branches
12. et al.

**Rootwork** - Zheng et al ICCV 2011

**RootReader3D** - Clark et al Plant Physiology 2011

**RSA-Gia pipeline** – Topp et al PNAS 2013



Capturing the **time dimension** is key to understanding how roots interact with the environment



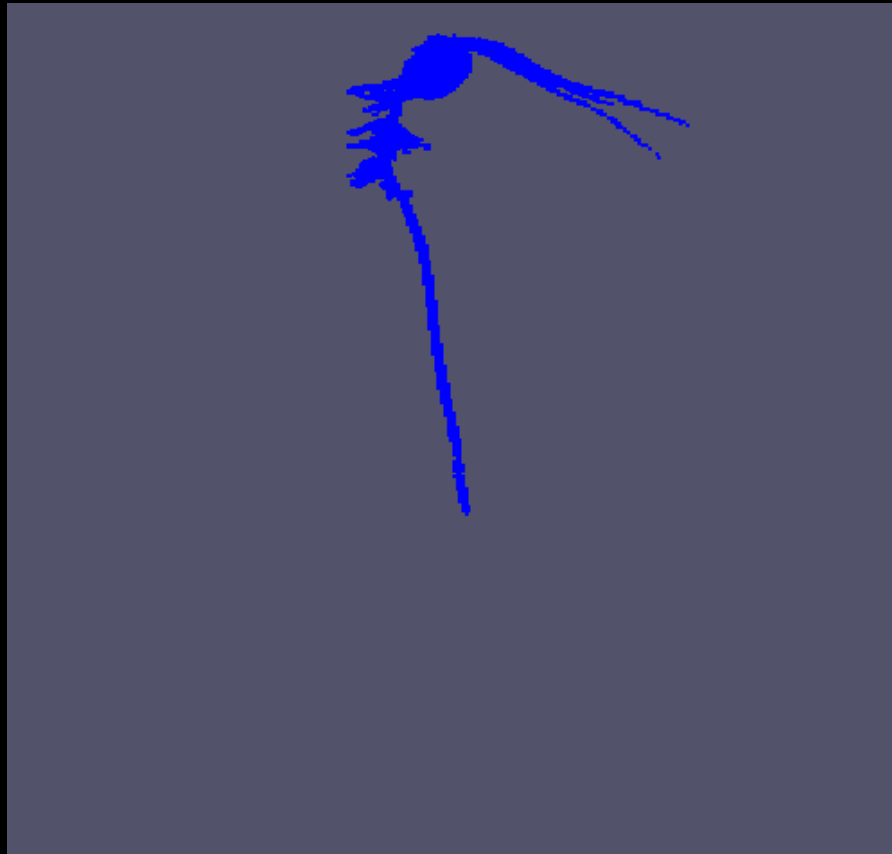
# DynamicRoots: 3D time series analysis software

For each root at each time point:

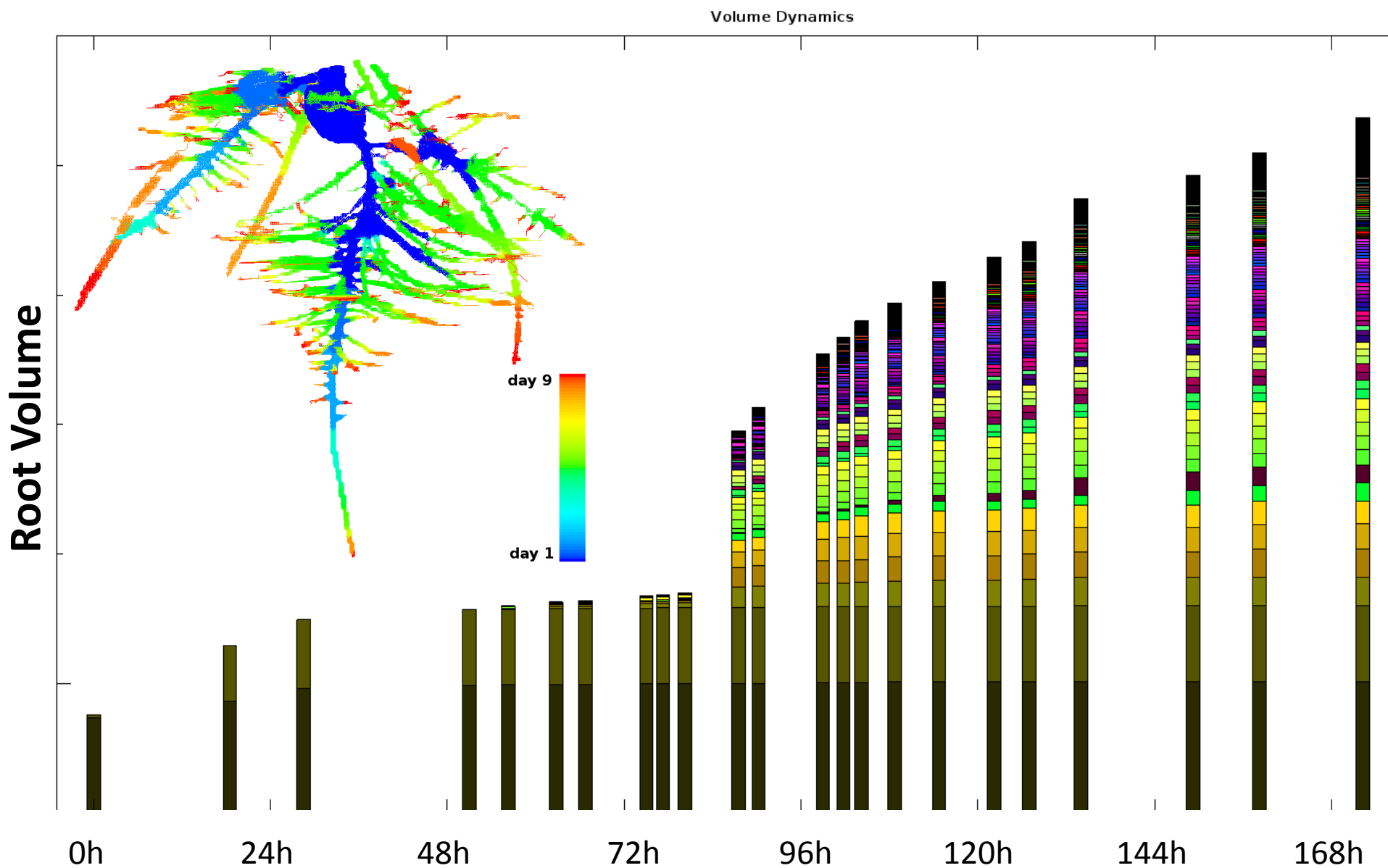
topological order, length, width, volume, angles, curvature, etc

For some or all roots at one time or as a function of time:

entire RSA topology, depth distributions, emergence and tip angles, dynamic curvatures, behavior in XYZ defined ROIs, etc.



# Dynamic Roots: per branch measurements of root growth over time



# What are the processes driving root growth under different environmental stimuli?

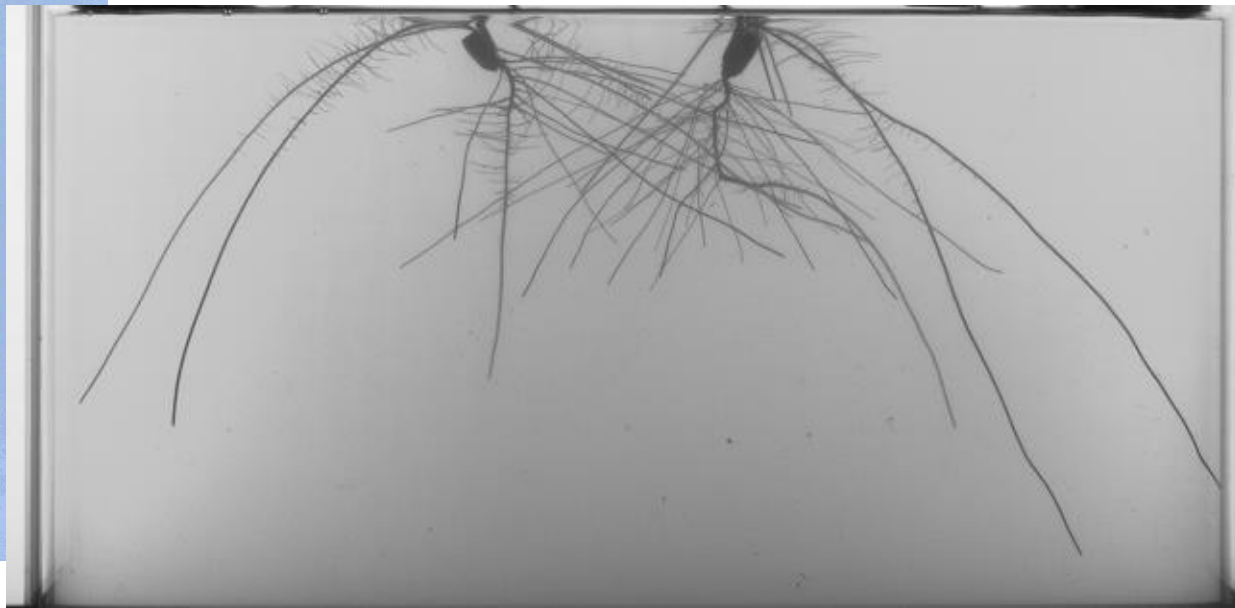


high Nitrogen

This image shows a single plant root system against a blue background. The root is relatively short and has a few small, lateral roots. A horizontal white line is drawn across the image, separating the 'high Nitrogen' section from the 'low Nitrogen' section.

low Nitrogen

This image shows a single plant root system against a blue background. The root is significantly longer and has many more lateral roots than the one in the 'high Nitrogen' section. A horizontal white line is drawn across the image, separating the 'high Nitrogen' section from the 'low Nitrogen' section.

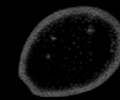
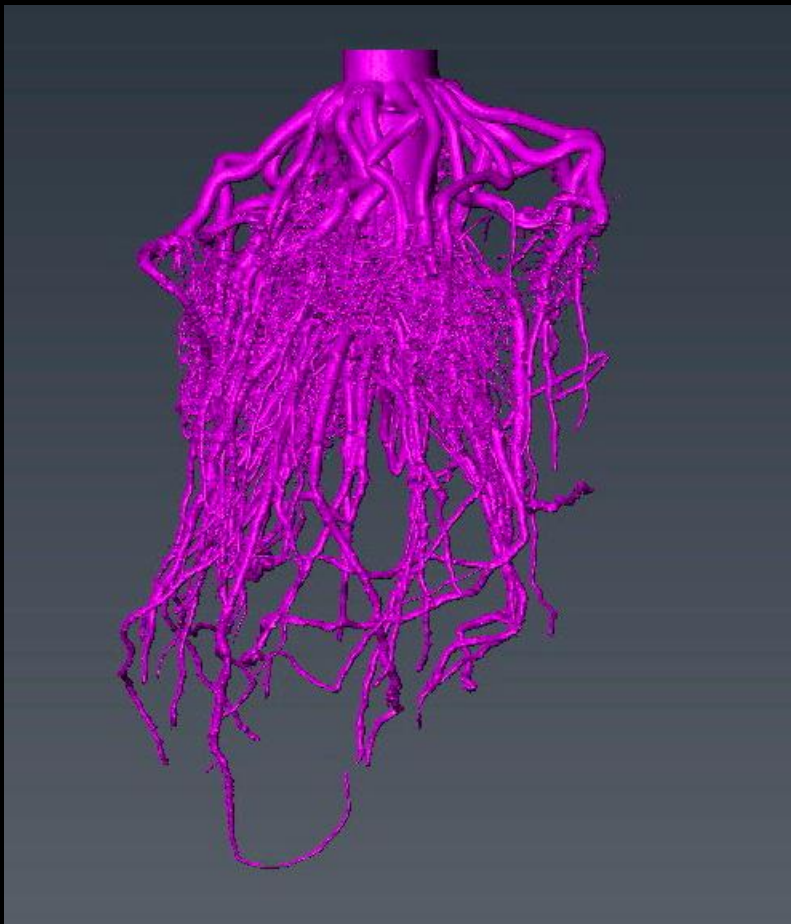




X-ray  
Computed  
Tomography:  
a medical and  
industrial  
workhorse  
that can be  
used for plant  
science



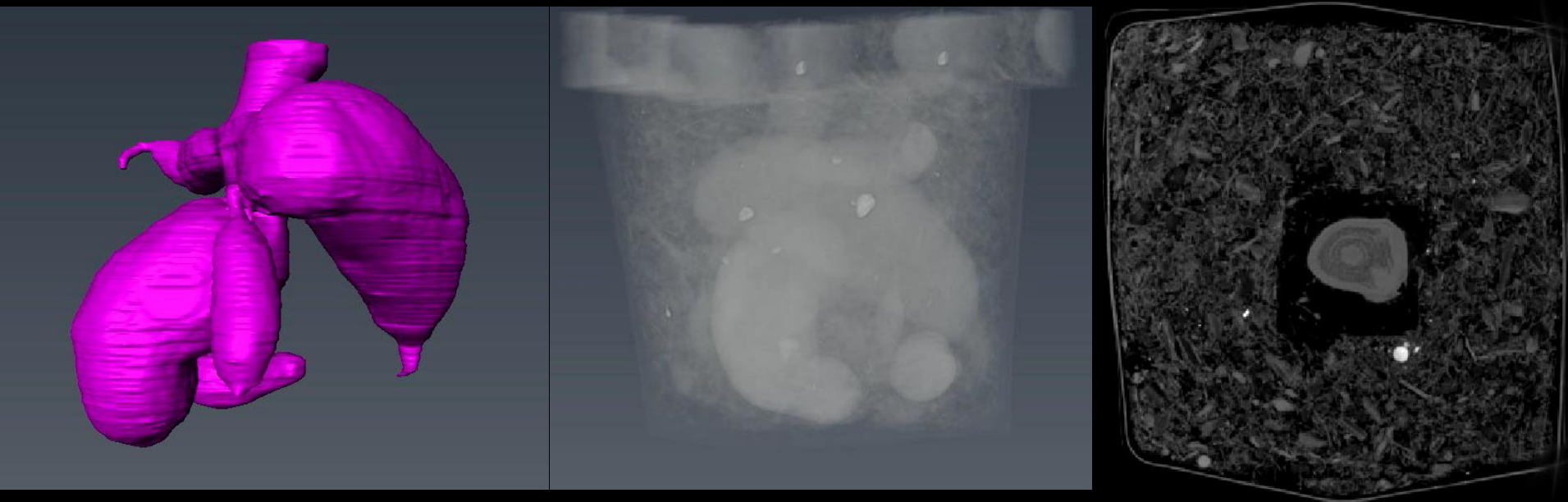
# X-ray CT can be used to extract fine-resolution information from complicated architectures in high-throughput



<b>Volume</b>	0.9 x 10 <sup>6</sup>
<b>Convex Volume</b>	14 x 10 <sup>6</sup>
<b>Solidity</b>	0.0661
<b>Depth</b>	433
<b>Total Length</b>	74,486
<b># tips</b>	1284

X-Ray CT scanned excavated root crown  
scan time ~ 2 minutes  
resolution 110 micron

X-ray CT can be used to quantify subterranean biomass in high throughput

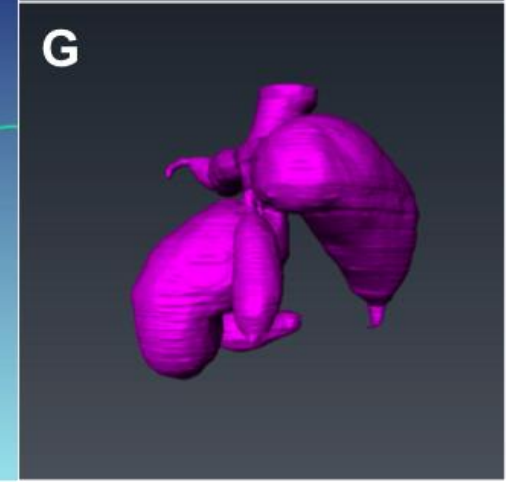
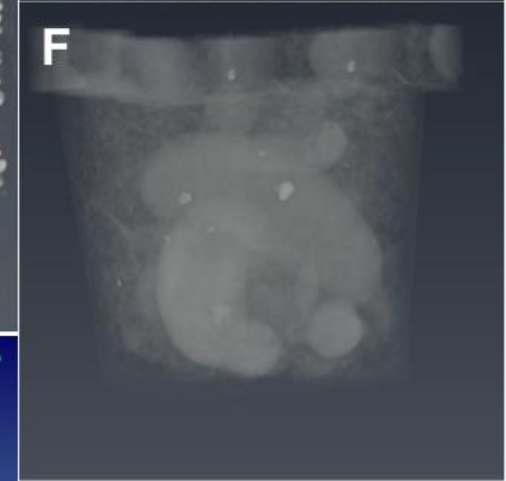
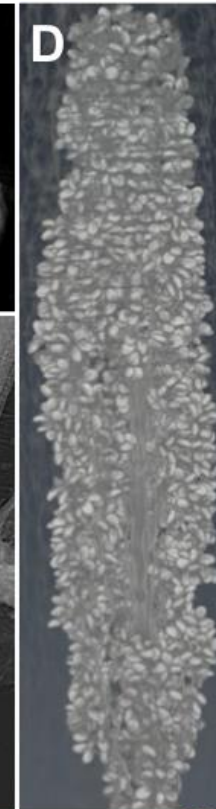
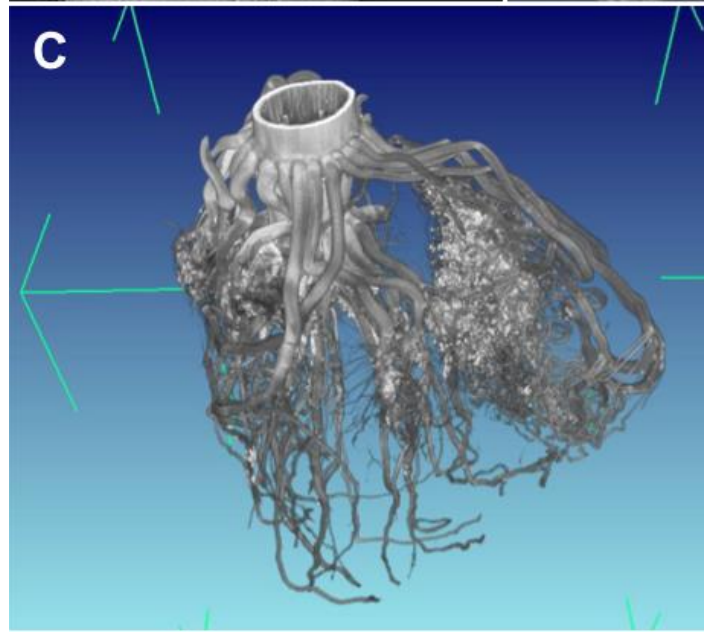
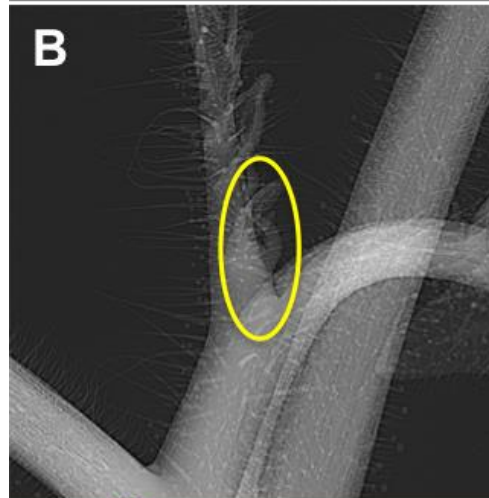
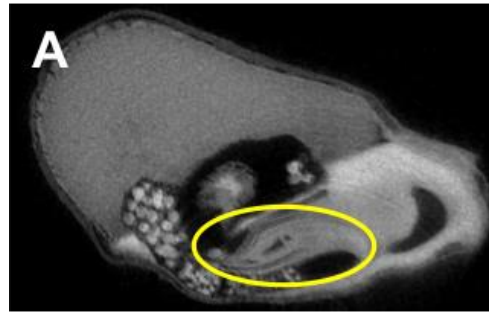


X-Ray CT scanned excavated cassava root | scan time ~ 5 minutes | resolution 110 micron

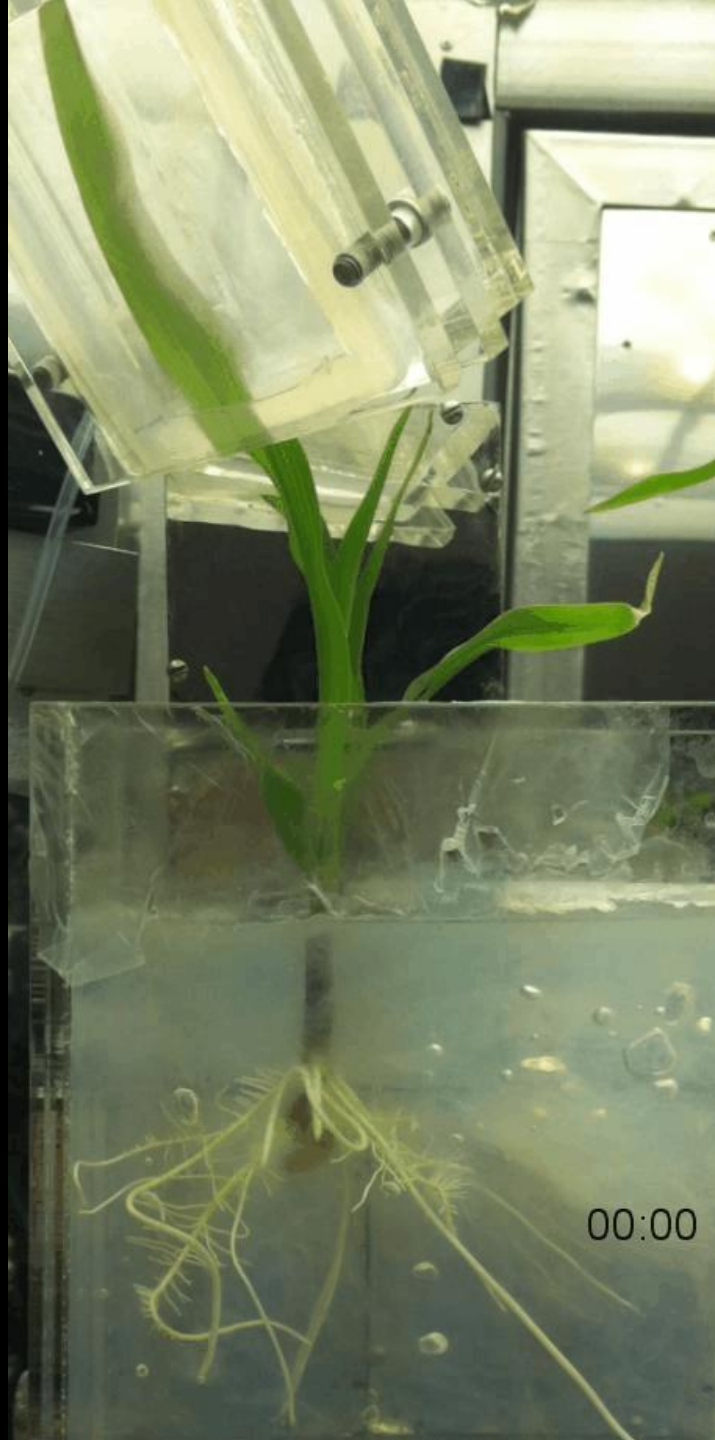
Volume	Convex Volume	Solidity	Depth	Total Length	# tips
$3.6 \times 10^6$	$10 \times 10^6$	0.3591	285	1792	6



**X-ray CT can be  
used to quantify  
whole plant  
morphology  
through the  
course of  
development at  
micro and macro  
scales**



**Positron Emission  
Tomography (PET):**  
to image whole  
plant carbon  
allocation and  
other dynamic  
physiological  
processes

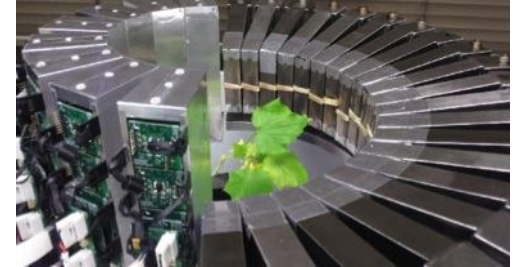


PhytoPET - 2013 IEEE  
S. Lee, B. Kross, J.  
McKisson, J.E.  
McKisson, A.G.  
Weisenberger, W. Xi,  
C. Zorn, G. Bonito,  
C.R. Howell, C.D. Reid,  
A. Crowell, L. C.  
Cumberbatch, C.  
Topp, and M.F. Smith

# Plant PET System

Funded by a NSF MRI Grant DBI-1040498

A cucumber plant labeled with  $^{11}\text{CO}_2$



PET imager integrated in a plant growth chamber

**Yuan-Chuan Tai**, Qiang Wang, Sergey Komarov,

Aswin J Mathews, Ke Li, Jie Wen,

**Joseph A O'Sullivan**

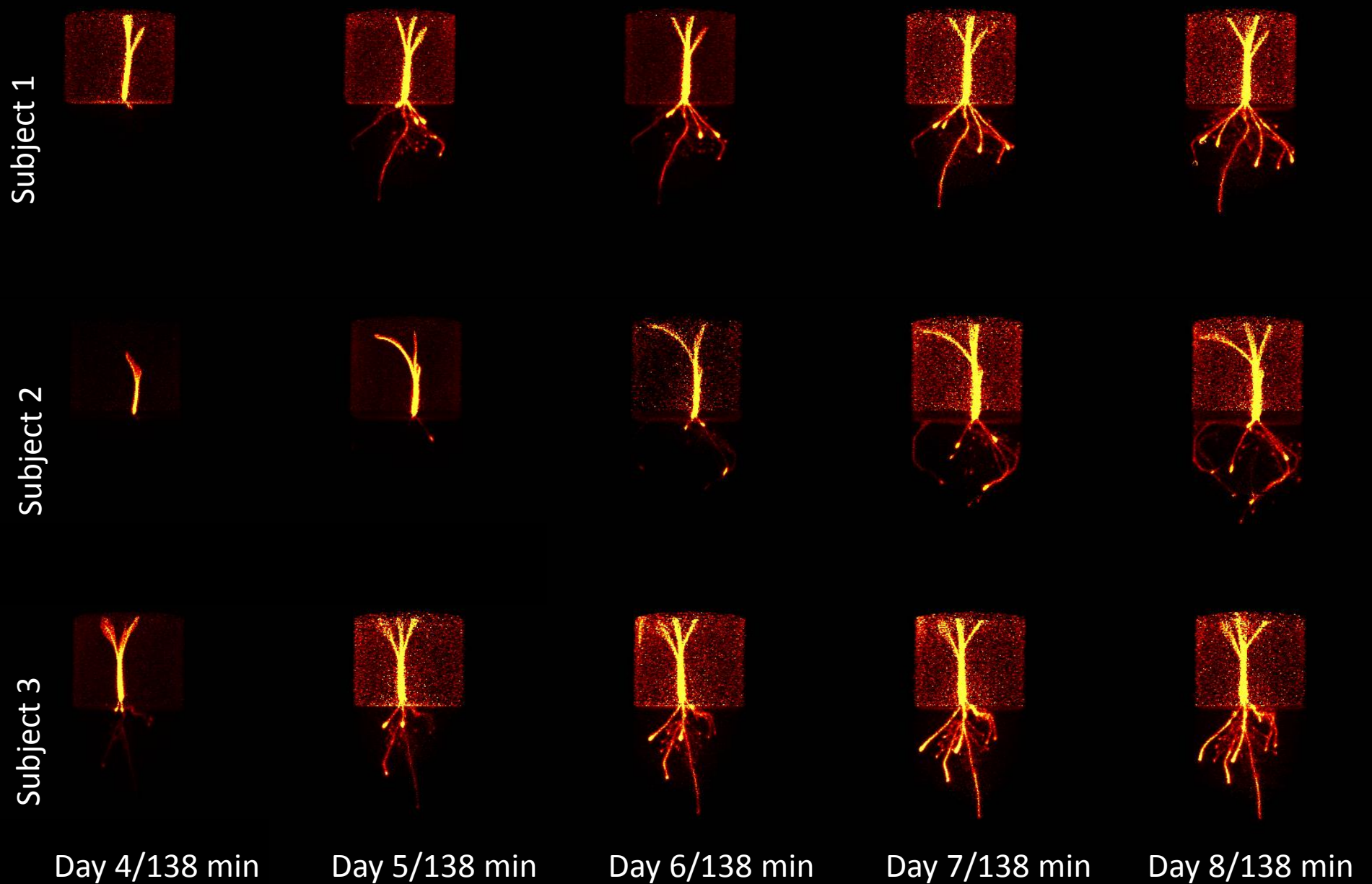
Washington University

Department of Radiology

Department of Electrical and Systems Engineering



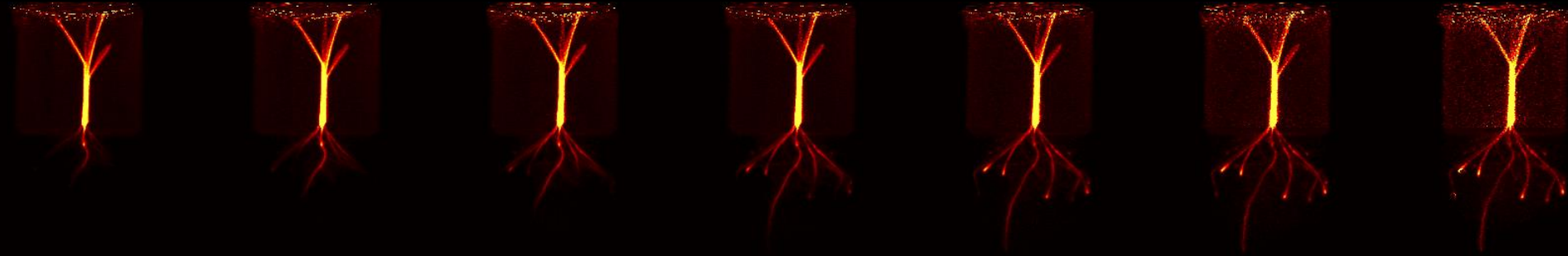
**Carbon allocation dynamics can be measured over multiple days of development**



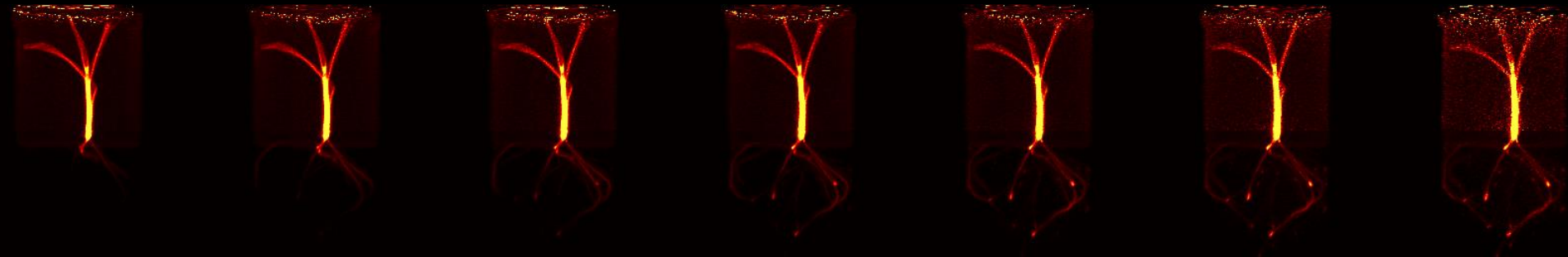


# Carbon allocation dynamics can be measured in real time

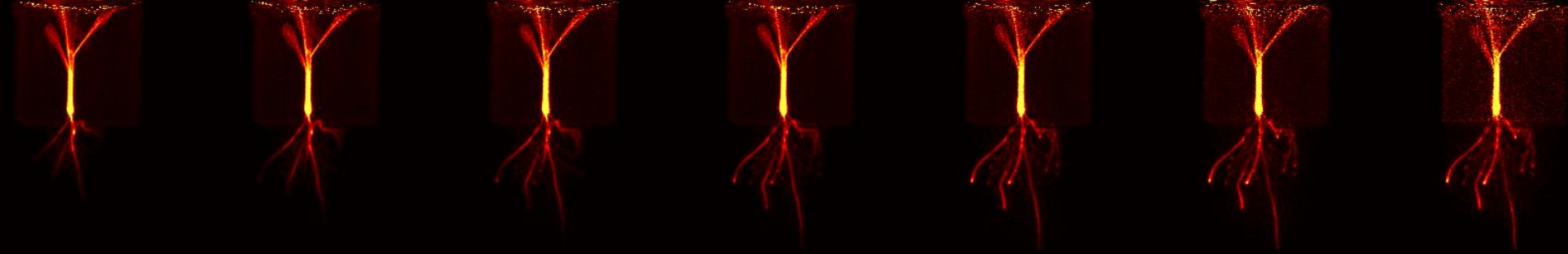
Subject 1 – Day 8



Subject 2 - Day 8



Subject 3 – Day 8



48 min

60 min

72 min

94 min

116 min

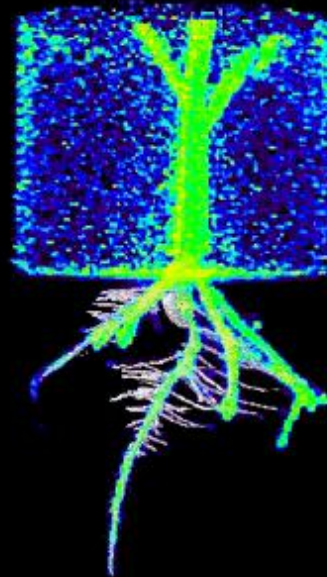
138 min

160 min

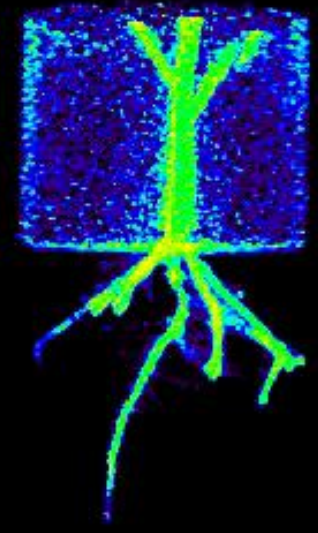
# Combined Optical Projection Tomography and PET (OPT-PET) imaging



3D OPT



Co-registered 3D PET/OPT

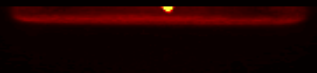


3D PET

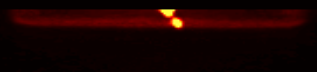
# no carbon allocated to seed-derived roots at day 03



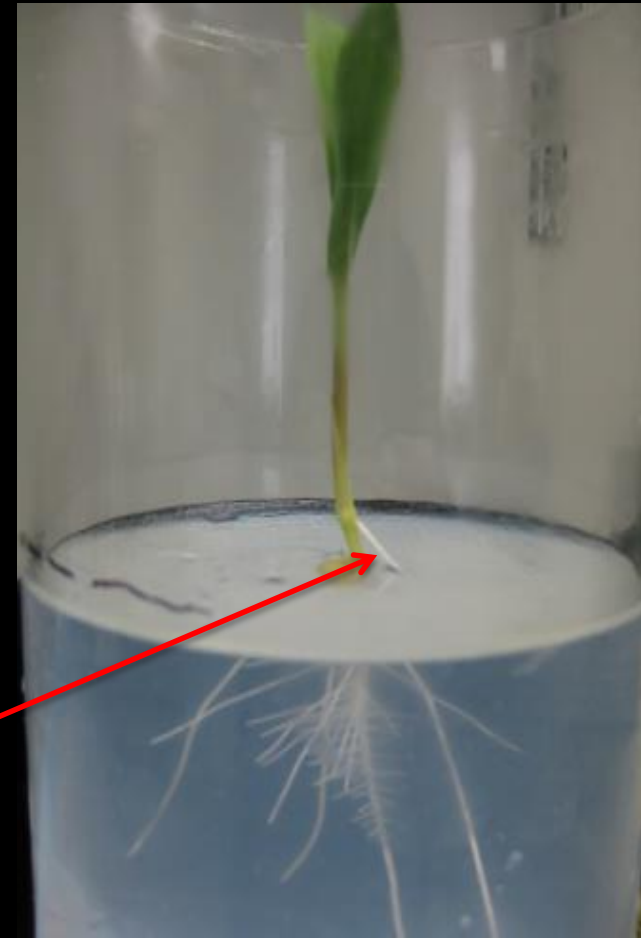
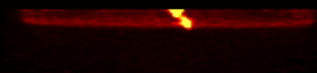
30 min



60 min

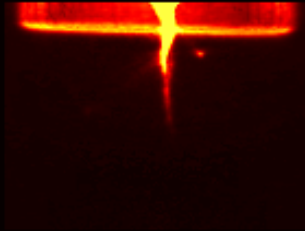


80 min

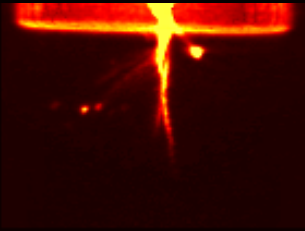


# carbon moves to seed-derived roots at day 04

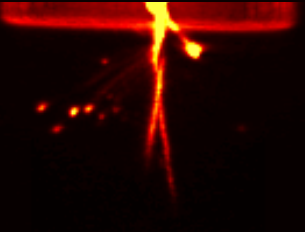
28 min



38 min

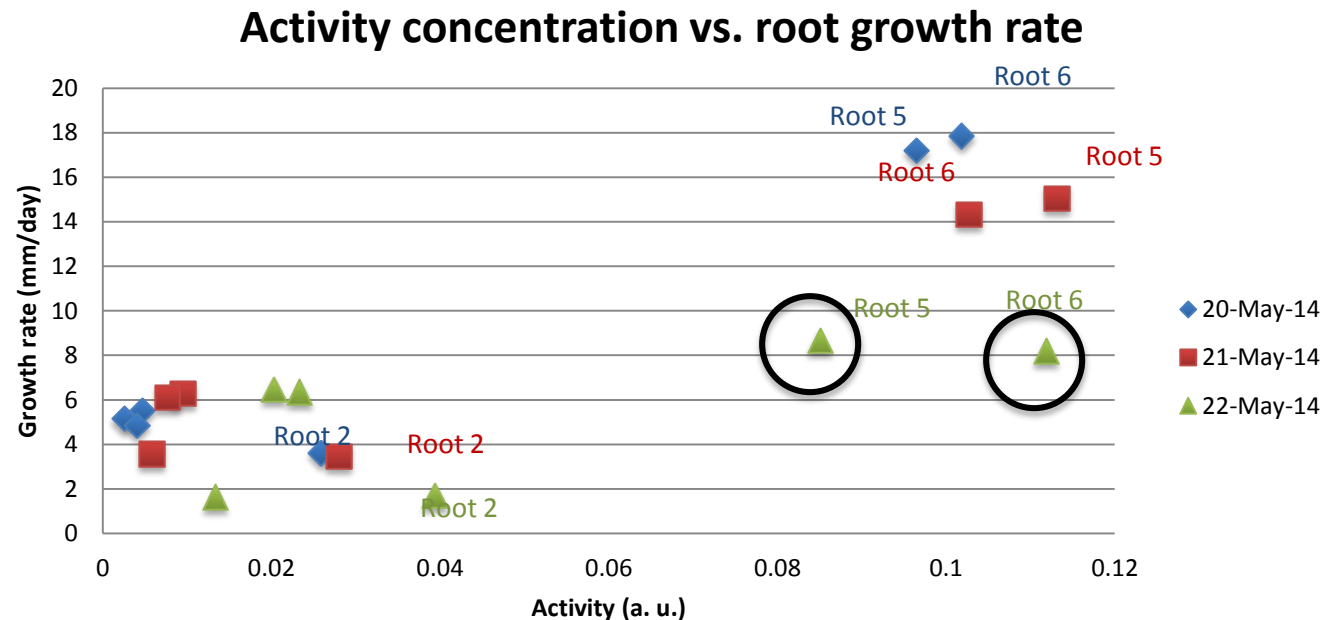
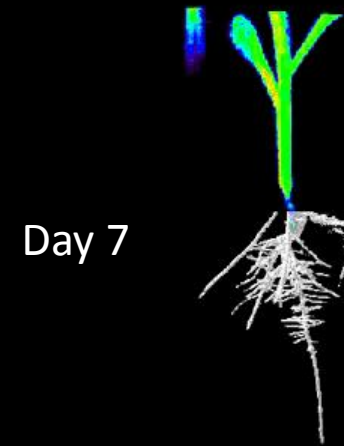
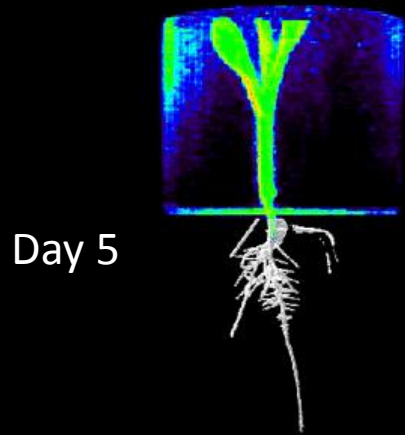


48 min



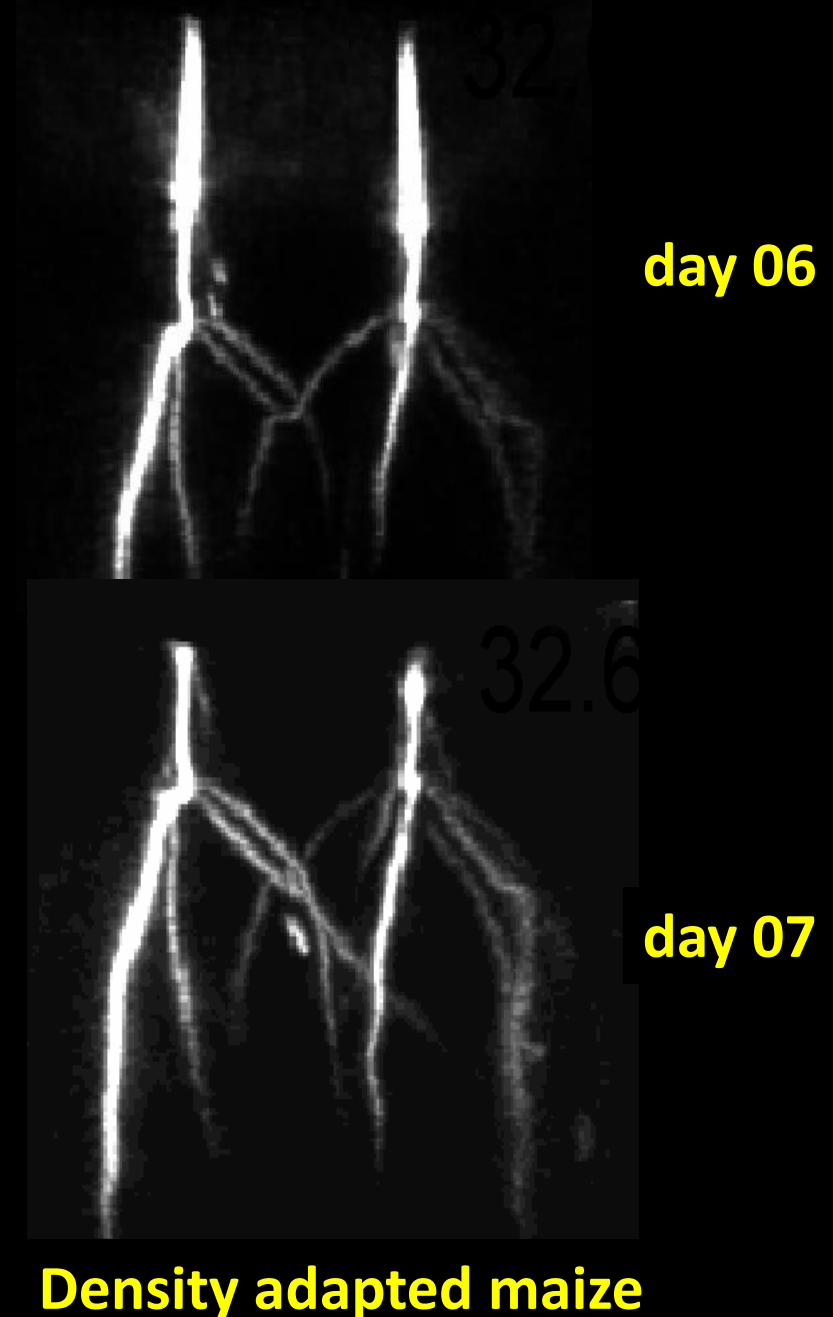


# OPT-PET can be used to measure root growth as a function of carbon allocated



Root growth rates are measured from OPT

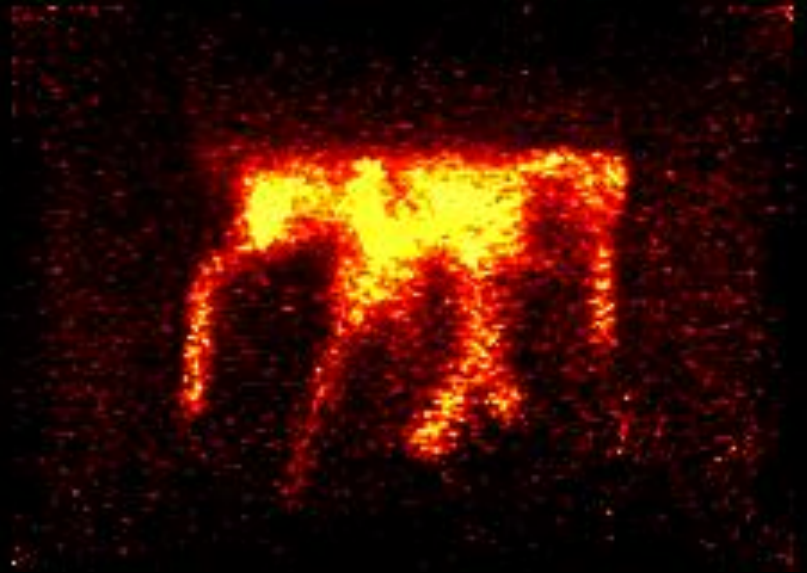
# OPT-PET can be used to quantify root-root and root-microbe interactions



# OPT-PET can be used to quantify root exudation



with maize plant



plant is pulled out

Day 05

punctate  
signals

Day 07

lateral  
roots

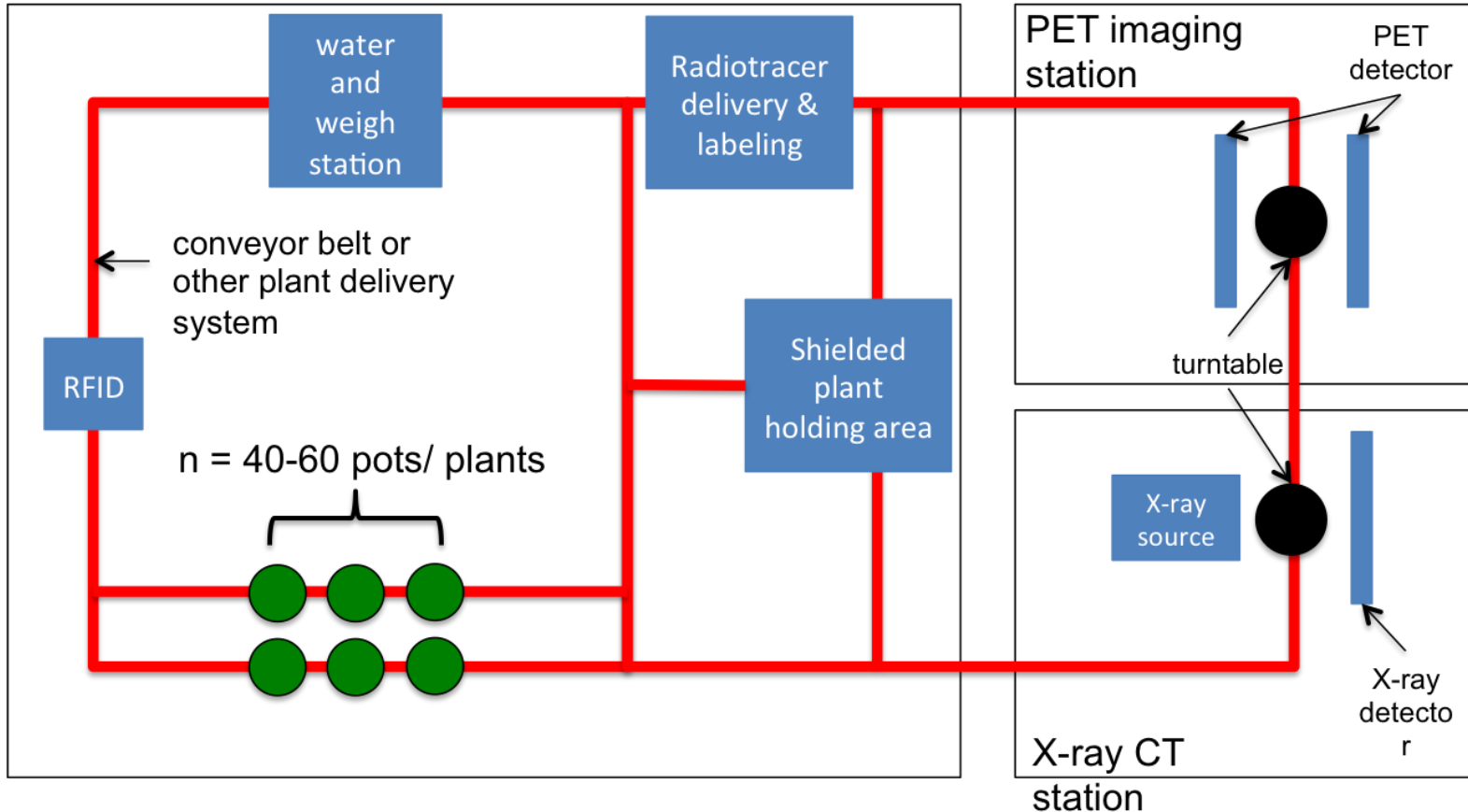
**OPT-PET:** using  
physiological  
signals to identify  
precise  
spatiotemporal  
patterns of  
morphological  
change



# Proposed XRT-PET plant imager

Controlled Environment Plant Growth Module  
(or equivalent, 100 sq ft)

Imaging Module (80 sq ft)



1. fully automated watering, weighing, sample tracking
2. automated PET radiotracer delivery and labeling system
3. PET imaging station(configurable geometry)
4. X-ray CT imaging station(configurable geometry)
5. imaging loop can run independently of water/weigh and radiotracer labeling
6. water/weigh can run independently of imaging loop

# How can we move beyond laborious, destructive field root phenotyping?



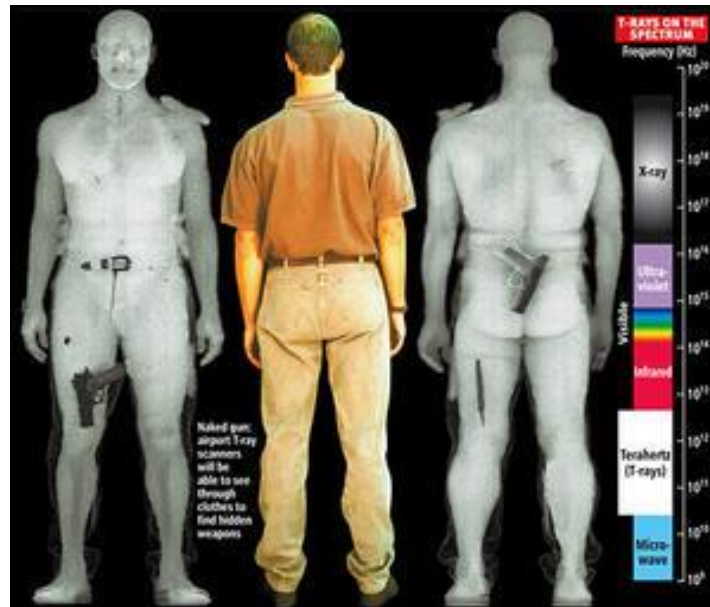
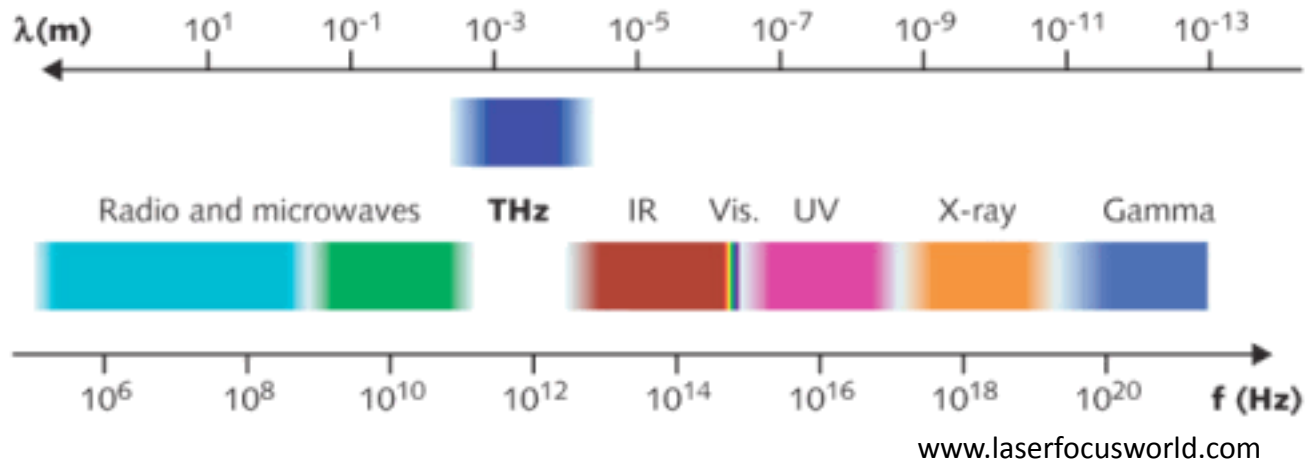
[serc.carleton.edu](http://serc.carleton.edu)

Large-scale minirhizotron mapping in conjunction with shovelomics/DIRT to map root architecture in the field



Project with Andrew Leakey, Ivan Baxter and Steve Moose

**Terahertz imaging** is an emerging non-destructive evaluation technique that can be used to identify objects of interest that are otherwise opaque in the visible light spectrum



Terahertz scanner imagery // Source: dailymail.co.uk

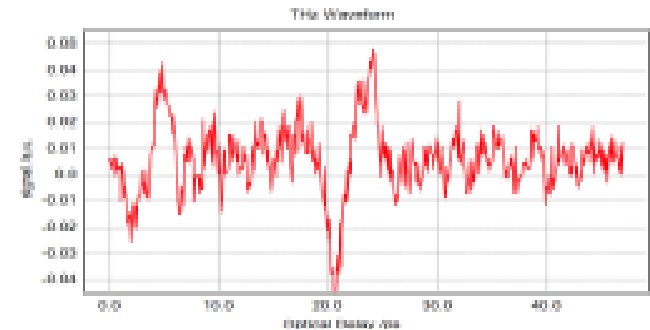
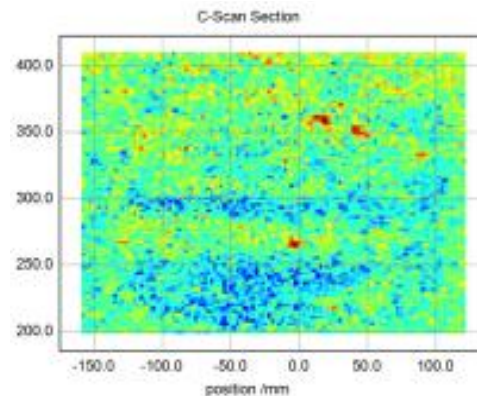


# Towards Root Phenotyping in-situ Using Terahertz Imaging

N. Smith<sup>1</sup>, N. Burford<sup>2</sup>, L. Rivera<sup>1</sup>, T. Bowman<sup>2</sup>, M. O. El-Shenawee<sup>2</sup>, and G. N. DeSouza<sup>1</sup>

<sup>1</sup>ViGIR - Vision-Guided and Intelligent Robotics Lab, University of Missouri

<sup>2</sup>Terahertz Imaging and Spectroscopy Lab, University of Arkansas



Classification Accuracy %	
Sand+Potato	92.50
Sand+Rock	94.68
Sand+Turnip	91.43
Sand+Wood	87.73

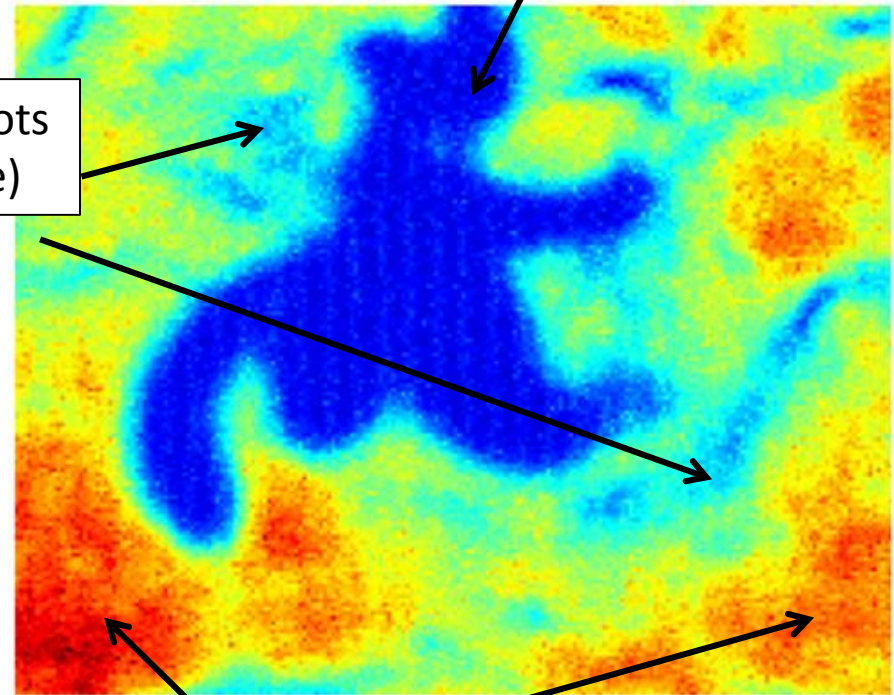
(a) Photo of the samples used for the THz reflection test including a sweet potato, a turnip, four rocks and a piece of tree branch (uncovered to show the objects); (b) Time domain THz reflection image of the objects after being completely buried by dry sand; (c) The time domain reflected signal at a particular point in (b); (d) Final classification using the HiGUSSS Framework

# Terahertz imaging of a cassava root system buried in sand



Smaller Roots  
(light blue)

Storage Roots  
(Dark Blue)



Sand (red-yellow)

**Guilherme DeSouza** - University of Missouri-Columbia | **Magda El-Shenawee** – University of Arkansas-Fayetteville | **Felix Fritschi** – UM-Columbia | **Nigel Taylor** – Danforth Center  
**Chris Topp** – Danforth Center

# Multidisciplinary Collaborators

## **Herbert Edelsbrunner Lab @ IST**

Olga Symonova

## **Joshua Weitz @ GA Tech**

Alex Bucksch

## **Yuan-Chuan Tai Lab @ WUSM**

Sergey Komorav

Qiang Wang

## **Dan Goldman Lab @ GA Tech**

Daria Moanenкова

## **Drew Weisenberger @ J-Labs**

Seungjoon Lee

## **Tim Horn @ NC State**

## **Mark Anastasio @ WUSTL**

Trey Garcon

## **Steve Moose Lab @ UIUC**

## **Jode Edwards @ USDA/ IA State**

## **Ivan Baxter Lab @ USDA/DDPSC**

## **Dan Chitwood Lab @ DDPSC**

## **Sherry Flint-Garcia Lab @ USDA/UMC**

Zhengbin Liu

## **Leon Kochian Lab @ Cornell**

Randy Clark (Pioneer)

## **Philip Benfey Lab @ Duke**

Anjali Iyer-Pascuzzi (Purdue)

## **Thomas Mitchell-Olds @ Duke**

Jill Anderson (USC)

Cheng-Ruei Lee (GMI)

## **Special thanks to:**

Bruce Hibbard and Tim Praiswater

Martin Bohn and Nicole Yana